Effect of *in Vivo* Administration of Various Oral Hypoglycemic Agents on Hepatic Protein Synthesis* (32704)

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Recent experiments have shown that the *in vitro* addition of either of two oral hypoglycemic agents, tolbutamide and phenethylbiguanide, would inhibit the incorporation of leucine-^{14}C into the protein of rat liver homogenates (1). It has further been reported that this *in vitro* inhibition is observed when isolated microsomes derived from normal rat liver are used at the protein synthesizing system (2,3). This agrees with reports by Manchester and Young (4) and by Jarrett and Butterfield (5) who observed that the *in vitro* addition of tolbutamide to rat diaphragm caused a decrease in the incorporation of alanine-^{14}C into the protein of the diaphragm.

On the other hand, Recant and Fischer (6) have reported that the oral administration of tolbutamide to rats increases the ability of liver slices to incorporate glycine-^{14}C into protein. Because of this reported difference between the *in vivo* and *in vitro* effects of tolbutamide on protein biosynthesis, it was considered necessary to investigate the *in vivo* effects more closely in order to understand better the action of the oral hypoglycemic agents.

Materials and Methods. Female albino rats of the Holtzman strain, fed *ad libitum* on standard laboratory chow, were used. The animals were injected intraperitoneally with an aqueous solution of the compound under study; control animals were injected with an equimolar amount of saline. In the acute studies, a single large dose of the compound was administered 2.5 hours before the animal was sacrificed. Animals were given either 0.35 mmoles of tolbutamide/150 g, 0.10 mmoles of phenethylbiguanide/150 g, or an equivalent amount of saline. The amount of phenethylbiguanide given was less than that of tolbutamide because of its greater toxicity. Whenever larger amounts of phenethylbiguanide were administered (i.e., 0.35 mmoles/150 g), the animal died within an hour. The cause of death appeared to be a fatal hypoglycemia. In the experiments utilizing chlorpropamide, it was administered in the same concentration as tolbutamide. The chronic studies involved the daily injection of animals with a smaller dose of compound (tolbutamide 0.10 mmole; phenethylbiguanide 0.02 mmoles) for a period of 4–8 days.

Following injection, the animals were sacrificed by decapitation and the livers quickly removed and immersed in ice-cold buffer. Microsomes were isolated by the procedure described by Campbell and Kernot (7). Incubations were performed for 2 hours at 37°C in an Elmac incubator-shaker apparatus. The details of the incubation procedure have been described previously (3). The reactions were terminated by the addition of 1.0 ml of 10% trichloroacetic acid (TCA) and the resulting precipitate was washed once with 5% TCA, three times with ethanol, and three times with acetone. The final washed precipitate was dissolved in 2.0 ml of 0.1 *N* sodium hydroxide and a 1.0 ml aliquot was taken to dryness and counted in a thin window Geiger–Mueller counter to a 5% probable error.

Results. Table I demonstrates the effect of the acute injection of either of three oral hypoglycemic agents upon the incorporation of L-leucine-^{14}C into the hepatic protein of normal rats. The injection of 0.35 mmoles of either tolbutamide or chlorpropamide results in a significant inhibition of protein biosynthesis. This inhibition is seen to be of the same magnitude for either of these two agents. Phenethylbiguanide likewise produces

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TABLE I. Effect of *in Vivo* Injection of Various Oral Hypoglycemic Agents on the *in Vitro* Incorporation of Leucine-^{14}C into Hepatic Protein of Normal Rats.

Injection given	Av cpm	No. of determinations
Saline 0.35 mmoles	2636	8
Tolbutamide 0.35 mmoles	1445	8
Chlorpropamide 0.35 mmoles	1711	4
Phenethylbiguanide 0.10 mmoles	1712	4

an inhibition of protein biosynthesis, but only 0.10 mmole of this compound is required to produce relatively the same degree of inhibition. These results conflict with those previously reported for a liver slice system.

Table II shows the results when smaller amounts of either tolbutamide or phenethylbiguanide were injected over a period of time. These smaller amounts of agents (0.1 mmole/day for tolbutamide or 0.02 mmole/day for phenethylbiguanide) produced no significant effect.

It is possible that the amount injected was too small to produce an effect; it is, however, more likely that the chemicals were metabolized before the incorporation study was made. This seems reasonable since the animals were sacrificed fully 24 hours after the final injection was given. If this latter explanation is correct, it might indicate that although these compounds do inhibit protein biosynthesis, they do not cause any irreversible damage to the synthesizing mechanism.

The experiments described in Tables III and IV represent an attempt to gain some insight into the mechanism of the inhibition. Animals were given an acute injection of either saline or a hypoglycemic compound. Two and one half hours after the injection, the animals were sacrificed and the microsomes and cell sap were isolated. A series of "crossover" incubations was now performed, in which one set of flasks contained both sap and microsomes derived from control animals; a second set received cell sap from control but microsomes from treated animals; a third con-

TABLE II. Effect of Repeated *in Vivo* Injections of Oral Hypoglycemic Agents on the *in Vitro* Incorporation of Leucine-^{14}C into Hepatic Protein.

Injection given	Dose of injection (mmole)	Duration of injections (days)	Av cpm	No. of determinations
Saline	0.1	4	10351	6
Tolbutamide	0.1	4	10562	6
Saline	0.1	8	2491	6
Tolbutamide	0.1	8	2475	6
Saline	0.02	4	8031	6
Phenethylbiguanide	0.02	4	7403	6
Saline	0.02	8	1550	4
Phenethylbiguanide	0.02	8	1871	5

TABLE III. Effect of *in Vivo* Injection of Tolbutamide on the Relative Ability of Microsomes and Cell Sap to Incorporate Leucine-^{14}C into Hepatic Protein.

Cell sap source	Microsomes source	Av cpm[a]		
		Trial I	Trial II	Trial III
Control animals	Control animals	3047	2447	1870
Tolbutamide animals	Control animals	2383	2049	1457
Control animals	Tolbutamide animals	3185	2556	1797
Tolbutamide animals	Tolbutamide animals	2559	2159	1425

[a] Each figure represents the mean of 3 determinations.

TABLE IV. Effect of *in Vivo* Injection of Phenethylbiguanide on the Relative Ability of Microsomes and Cell Sap to Incorporate Leucine-^{14}C into Hepatic Protein.

Cell sap source	Microsomes source	Av cpm[a] Trial I	Trial II
Control animals	Control animals	2528	2416
PEBG animals	Control animals	1810	1489
Control animals	PEBG animals	2563	2218
PEBG animals	PEBG animals	1790	1372

[a] Each figure represents the mean of 3 determinations.

tained cell sap from treated animals but microsomes from control animals; the final set received both cell sap and microsomes from the treated animals. Table III shows the results when the animals were injected with tolbutamide and Table IV when phenethylbiguanide was the compound employed. In all cases, an inhibition is observed when the cell sap from treated animals is used in the incubation, and no inhibition is ever seen when only the microsomes are derived from the treated animals. Further, the inhibition seen when both cell sap and microsomes are derived from treated animals is no greater than that observed when only the cell sap is obtained from the treated animals. Clearly then, the cell sap is the important constituent as regards the inhibition of protein synthesis seen by the *in vivo* injection of either tolbutamide or phenethylbiguanide. There are several possible explanations for this phenomenon. The most obvious interpretation is that the site of action of the oral hypoglycemic agents lies within the cell sap, i.e., these agents interfere with either the activation of amino acids or with their transfer to soluble RNA. It is also possible, however, that these agents really act at the microsomal level, and are merely carried in the cell sap. If this were the case, the addition of cell sap to an incubation flask from a treated animal would be the same as the addition of a dilute solution of the compound. In either event, the data does demonstrate that neither agent binds to the microsomes—both either act in the cell sap or are carried in the cell sap.

Discussion. Experiments in which a compound is injected into an animal *in vivo* are far more complex in regard to interpretation than simple *in vitro* experiments because a number of conflicting factors might contribute to the final result. For example, the injection of tolbutamide into an animal might be expected to produce at least two antagonistic results: the tolbutamide itself would inhibit the biosynthesis of protein as previously demonstrated in *in vitro* systems; and the tolbutamide would act on the pancreas of the animal to stimulate the production of insulin which in turn would accelerate the biosynthesis of protein. The net effect observed would depend upon which of these individual effects was of the larger magnitude.

The results reported in this paper indicate that the injection of either tolbutamide or phenethylbiguanide may result in an impaired ability of the animals liver to incorporate amino acids into protein. Under these conditions (i.e., a rather large injection of the agent under consideration) it seems apparent that the direct inhibitory effect of the compound is paramount and overshadows any secondary effects which might tend to promote protein anabolism.

When smaller amounts of the agents were administered over a period of time (Table II), no net effect upon the *in vitro* incorporation of amino acids into hepatic protein was observed. Under these conditions, the inhibitory effect of the compounds might be compensated for by other secondary anabolic effects.

It is likewise significant that in no case studied did either tolbutamide or phenethylbiguanide cause a net stimulation of protein synthesis, as has been repeatedly demonstrated following the injection of insulin (8). This implies that these agents do not possess all the attributes of insulin and might not be as effective as insulin in controlling the entire diabetic state.

Summary. The effects of the *in vivo* injection of two oral hypoglycemic agents (tolbutamide and phenethylbiguanide) upon the *in vitro* incorporation of leucine-^{14}C into hepatic protein were measured. The acute injection of a large amount of either agent impaired the ability of the animals liver to synthesize protein. This impairment was seen to occur at the level of the cell sap and was

not associated with the microsomes. The daily injection of smaller amounts of these compounds resulted in no significant effect on protein metabolism. The data appear to indicate that these agents may, under certain circumstances, have a deleterious effect on the protein balance of the organism.

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Thymic Control of Cellular Differentiation in the Immunological System* (32705)

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In mice, removal of the thymus at birth results in lymphopenia and deficient immunological responses (1). Similarly, when the immunological system of animals thymectomized in adult life is damaged by irradiation, recovery of immune responsiveness is impaired when compared with that of intact, irradiated controls (1). However, little is known about the precise functions of the thymus at the cellular level. The results of recent studies on the various classes of cells responsible for immune responses now make it possible to examine the stage in differentiation for which the presence of the thymus is essential. Differentiation in the immunological system is based on at least three classes of cells. The most differentiated class consists of antibody-producing cells which arise by proliferation and differentiation from antigen-sensitive precursors (2,3). Antigen-sensitive cells appear to have limited proliferative potential (4), suggesting that their numbers may be replenished from a yet more primitive precursor. Evidence for the existence of such precursors has been obtained from studies of bone marrow and fetal liver. These tissues contain no demonstrable antigen-sensitive cells (4); nonetheless, when bone marrow or fetal liver cells are given to heavily irradiated mice there is eventual recovery of immunological responsiveness (5,6). Thus, bone marrow and fetal liver contain a class of precursors which are not sensitive to antigen, but which have the capacity to differentiate, giving rise to antigen-sensitive cells.

Mice thymectomized at birth contain very few antigen-sensitive cells (7). However, when antigen-sensitive cells from normal mice are injected into neonatally thymectomized mice they yield a normal number of plaque-forming cells (8). This experiment indicates that the thymus plays little, or no role in the differentiation of antigen-sensitive cells to plaque-forming cells. My experiments were designed to determine whether or not the thymus plays a role in the differentiation of bone marrow precursors to antigen-sensitive cells.

A direct assay for the marrow precursors of antigen-sensitive cells is not available. Therefore, these precursors were studied indirectly by injecting normal bone marrow into heavily irradiated mice and observing the appearance of their antigen-sensitive descendents in these recipients at varying intervals of time after marrow transplantation. The role of the thymus was assessed by comparing thymectomized, and intact, irradiated mice in this system.

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